Contents

S. Mitchell Harman, M.D., Ph.D., Acting Chief

Laboratory of Clinical Investigation

Gerontology Research Center
Room 2-B-20
Phone 410-558-8186
Fax 410-558-8346

The Laboratory of Clinical Investigation (LCI) chiefly focuses on clinical research issues of importance in gerontology. Much of the clinical work deals with volunteers on the Baltimore Longitudinal Study of Aging (BLSA), but additional clinical research protocols are also ongoing.

The **Applied Physiology Section** (APS) is concerned primarily with the physiology and pathophysiology that links normal changes in aging with two of the most common diseases of the elderly, osteoporosis and osteoarthritis. These diseases are among the most common causes of morbidity in the elderly population and their costs, both economic and personal, have been well documented. The effort in osteoporosis research on the BLSA volunteers pointed out the need for studying women who will be going through the menopause, and following the changes in bone mineral, hormones, lipids, and body composition that they will undergo as they traverse the menopause. This led to the third major effort in the section with the collaboration of our colleagues, especially in the Endocrinology Section and the Longitudinal Studies Section, the Perimenopausal Initiative. An additional effort that involves both osteoporosis and osteoarthritis is the cross-cultural investigation of these diseases in genetically, geographically, and culturally diverse populations, and comparing results around the world with those of the BLSA volunteers. In all these efforts we have used an epidemiological approach to identify risk factors associated with the diseases, biological measurements to ascertain the importance of hormones and biomarkers in the pathophysiology of these processes, physiological measurements to determine correlates with these processes, and genetic epidemiology to measure the familial components. Through the better understanding of the normal and pathological processes involved, we would hope to be able to develop a strategy for disease prevention and optimization of function in the elderly.

The **Diabetes Section** (DS) focuses on improving present methods for treating type 2 diabetic patients. Diabetes mellitus is one of the most prevalent diseases among the elderly. Approximately 40% of all adults

over the age of 65 have diabetes or elevated fasting glucose. Diabetes is also a comorbid condition in other conditions of the elderly, especially cardiovascular disease. By definition, diabetes mellitus is a group of metabolic diseases characterized by high blood sugar resulting from defects in insulin secretion, insulin action, or both. Type 2 diabetes is characterized by both defects. It is generally accepted that it is the elevated sugar which leads to the complications of diabetes. Therefore, we in the Diabetes Section feel that our endeavors should be directed towards improving insulin secretion or restoring insulin action. Despite the fact that 3 new agents have become available in the past eighteen months to treat type 2 diabetes, they have proven less than adequate at normalizing blood sugars.

The **Endocrinology Section** (ES) conducts and facilitates (by collaboration with other intramural and extramural entities) research aimed at understanding the particulars of changes in regulation of hormones during the normal aging process. It explores the relationships of hormone secretion to states of nutrition and health and the interrelationships among various hormone axes during aging. ES elucidates the influence of alterations of endogenous hormone activity on risk factors for susceptibility to chronic diseases associated with aging. Current efforts focus on changes in the growth hormone and reproductive hormone (sex steroids) axes. Finally, the ES conducts research investigating the clinical utility and risk/benefit ratios of rationally selected hormone replacement interventions, designed to reverse documented age-related alterations of hormone balance.

The **Longitudinal Studies Section** (LSS) has a twofold mission. The first is to manage the operations of the Baltimore Longitudinal Study of Aging (BLSA), a multidisciplinary longitudinal study of human aging. Research on aging using this open panel of research volunteers is performed by scientists based in several NIA intramural research laboratories and numerous outside collaborators. The second is to perform research with the BLSA using both existing data and data from newly initiated projects.

BLSA Operations: LSS staff schedules and manages the activities of the men and women research volunteers during their biannual two and half-day visits during which time the volunteers participate in numerous research studies. LSS staff conducts the clinical evaluations that establish health status of all active participants on every visit. The results are used in many investigations and also are used to determine the safety of research procedures for various participants. The results of the clinical evaluations are given to participants and to their physicians if requested by the participant.

Between visits, LSS staff maintain communication with participants, provide information about the findings of the study to participants both individually and by means of a periodic participant newsletter. They also maintain periodic contact with those who either are unable or unwilling to come in for regularly scheduled visits. LSS staff manages the recruitment of new research volunteers from a large group of applicants on a waiting list. LSS staff employs numerous mechanisms to learn about deaths in the study sample, obtain information about deceased BLSA participants and manage the autopsy program.

BLSA Research: LSS was given the responsibility to analyze, report and recommend continuing, changing or stopping a number of existing research projects without active investigators. Most had been started in the 1960s or 1970s and had either been recently discontinued or were ongoing. Project areas for which longitudinal analyses and reports were completed included: pulmonary function; hearing and vision, reaction time, reciprocal movement speed, nerve conduction velocity, power and strength measurements, self-reported participation in physical activities, blood pressure, and a variety of studies using clinical data.

New studies were initiated in the areas of prostate aging and disease, neuromuscular changes with age, hearing, physical functioning and disability and age differences in the dynamics of cerebral blood flow. All were designed to take advantage of the unique BLSA longitudinal database and all required the development of research teams from other laboratories and outside collaborators.

LSS staff developed a number of statistical approaches that facilitated the analysis of longitudinal data and have applied these approaches to a number of historical data sets in the BLSA.

The **Metabolism Section** (MS) has played a critical role in evaluating diagnostic standards and in determining whether an adjustment for age is appropriate. In two areas, diabetes and obesity, the standards in general use to define these diseases have not been age-adjusted during the adult years of life. The primary technique used to establish standards has been the relationship between levels (fasting glucose and glucose tolerance for diabetes and the Body Mass Index for obesity) and the subsequent development of complications that are strongly related to the diseases. The BLSA and the Follow-up Study of the National Health and Nutrition Examination Survey-I have provided unparalleled data sources for this effort. In both areas, the analyses suggest that adjustment of standards for age is required. In further studies in collaboration with other intramural and extramural scientists, factors influencing glucose/insulin homoeostatic mechanisms and quantification of the obese state are under study.

Laboratory of Clinical Investigation Staff

Office of the Chief

S. Mitchell Harman	Acting Chief
Irene Vasilios	Secretary

Applied Physiology Section

Jordan Tobin	Chief
Tracey Roy	Biologist

Diabetes Section

Josephine Egan	Acting Chief
Michele Buckler	Secretary
Michel Bernier	Senior Investigator
Chahrzad Montrose	Investigator
Lisa Adams	Biologist
WhaSeon Kwon	IRTA
Michael Garant	IRTA
Buel Rodgers	IRTA
Huan Yang	IRTA
Jie Zhou	Visiting Fellow
Yihong Wang	Visiting Fellow
Deokbae Park	Visiting Fellow
Thuan Nguyen	Student Support IRTA

Endocrinology Section

S. Mitchell Harman	Chief
Sue Feehley	Secretary
Dorothy Bertak	Medical Tech.

Longitudinal Studies Section

James L. Fozard	Chief
Audrey A. Molle	Office Automation Assistant
Karen M. Harris	Office Automation Clerk
E. Jeffrey Metter	Medical Officer
Barbara S. Hiscock	Program Analyst
Yoji Nagai	Visiting Fellow
Nicole Lynch	Pre-IRTA Fellow
Catherine Dent	Testing Manager
Claudia B. Willey	Program Coordinator
Gloria Hammen	Computer Technician
Sandra A. Pegram	Computer Technician
Ryan M. Warrenfeltz	Data Transcriber

Metabolism Section

Reubin Andres	Chief
Denis Muller	Computer Prog. Analyst
Howard Baldwin	Chemist
Mary Bannon	Biologist
Faye Barrack	Bio Lab Tech
Jarad Buchanan	Student Support IRTA

Jordan D. Tobin, M.D.
Chief, Applied Physiology Section

Gerontology Research Center
Room 3-C-01
Phone 410-558-8192
Fax 410-558-8318
E mail jordan_tobin@nih.gov

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Recent Publications:

Hirsch R, et al. *Ann Rheum Dis* 1996; 55: 25-29.

Muller DC, et al. *Aging Clin Exp Res* 1996; 8: 13-21.

Martin K, et al. *J Rheumatol* 1997; 24: 702-707.

Lethbridge-Cejku M, et al. *J Rheumatol* 1996; 23: 1943-1947.

Biography: Dr. Tobin received an A.B. from Columbia College in 1959 and an M.D. from New York University School of Medicine in 1963. He trained in Internal Medicine at Bellevue Hospital and in Diabetes at NIH with Dr. Andres from 1965-1967, and as an NIH Special Fellow at the EP Joslin Research Labs from 1967-1969. In 1969, he returned to the NIH to the Metabolism Section of the Laboratory of Clinical Physiology of National Institute of Child Health and Human Development (NICHD), later NIA. In 1981, he became Chief of the Human Performance Section and in 1986, Chief of the Applied Physiology Section of the Laboratory of Clinical Physiology. He is a past Chair of the Clinical Medicine Section and past President of the Gerontological Society of America.

The Applied Physiology Section (APS) is primarily concerned with the physiology and pathophysiology that links normal changes seen with aging with two of the most common diseases of the elderly, osteoporosis and osteoarthritis. These diseases are among the top causes of morbidity in the elderly population and their costs, both economic and personal, have been well documented. The effort in osteoporosis research on the BLSA volunteers pointed out the need for studying those women who will be going through the menopause, and following the changes in bone mineral, hormones, lipids, and body composition that they will undergo as they traverse the menopause. This led to the third major effort in the section with the collaboration of our colleagues, the Perimenopausal Initiative. In all these efforts, we have used an epidemiological approach to identify risk factors associated with the diseases, biological measurements to ascertain the importance of hormones and biomarkers in the pathophysiology of these processes, physiological measurements to determine correlates with these processes, and genetic epidemiology to measure the familial components. Through the better understanding of the normal and pathological processes involved we would hope to be able to develop a strategy for disease prevention and optimization of function in the elderly.

Osteoporosis: The APS effort in osteoporosis research includes studies on the BLSA volunteers, as well as specific populations. These include sunlight deficient elderly, and in collaboration with the Endocrine section, studies on elderly volunteers with low sex hormones and IGF-I who are part of a double-blind study of sex and growth hormone treatment. Our approach is multifactorial, with the assessment of bone mineral density at multiple sites being the central parameter, and physiological (i.e., strength, obesity, metabolism), biochemical (ionized calcium and markers of bone turnover), hormonal (PTH), and vitamin (Vitamin D and D3) parameters measured to relate to bone status and rates of bone change. The recognition that bone loss occurs in males as well as in females is an important aspect of this work, and the potential for increased morbidity from hip fractures in males becoming more important as more men live to an age at which hip fracture (the incidence rate is approximately half that in women) is more common. The understanding of the processes responsible for the age-associated bone loss is important for differentiating this loss from the disease osteoporosis if one is to be able to suggest preventive and treatment strategies. Future work includes the longitudinal assessment of biochemical and hormonal changes now that a sufficient number of volunteers have had repeat visits, as well as the longitudinal assessment of rates of change in bone density. These are both extensions of the initial cross-sectional work.

Osteoarthritis: The research on osteoarthritis (OA) in the BLSA started with an epidemiological description of the prevalence of hand OA in the original male cohort, and was expanded to include a longitudinal study of the development of hand OA in males and females, as well as the development of an axis of radiographic changes of individual features of the disease. Recent work has focused on the epidemiology of knee OA, as well as the development of an atlas of radiographic knee changes, the familial association of OA, the association with hand OA (polyao), its relationship to symptoms of pain, and biochemical and hormonal, physiological, and life style risk factors associated with abnormal xrays. Genetic studies of OA have included both epidemiological and molecular approaches. The familial association of OA of the hand and polyarticular OA was demonstrated in the family members of the BLSA with clinically relevant correlations between brothers, sisters, and sister-brother pairs for OA in each of the hand joints, in multiple joint site OA in the hands, and in polyarticular OA (including the knee joint). On a molecular level, we examined the association between alleles of the polymorphic gene controlling aggrecan protein, one of the most common proteins associated with cartilage, and bilateral hand OA. Men who have one of the most common alleles of this aggrecan gene have a five-fold greater likelihood of having moderate to severe hand osteoarthritis in multiple joints on both hands then men who do not.

Perimenopause: On the average, a woman in this country experiencing the menopause still has more than one-third of her life ahead of her. The development of cardiovascular disease, the leading cause of death in U.S. women, and osteoporosis and musculoskeletal impairment, common conditions that predispose to frailty in older women, is accelerated in the menopausal state. Cross-sectional studies suggest that the menopausal transition is associated with changes in the endocrine-metabolic milieu, body composition, uterine function, cardiovascular risk profile, and psychosocial well being. In 1993, the BLSA initiated a study of the perimenopause by starting to recruit a cohort of 100 White and 100 African-American women 45-55 years old. All recruits are healthy, nonsmoking women who are experiencing monthly menses at enrollment and who do not receive hormonal therapies. In addition to the bi-annual inpatient BLSA visits, these women receive quarterly outpatient visits until menses have ceased for 2 years, or hormone replacement is begun. These visits include a menopausal symptom questionnaire, endocrine profiles, anthropometry, dual energy x-ray absorptiometry, bone biochemistries, and psychosocial assessments. To date, 91 White women and 10 African-American women have been enrolled in the BLSA and have been followed from 3 to 45 months. Results on 21 women seen at least 3 times who were clearly premenopausal, showed no significant changes in the percent of their total body that was fat, their waist circumference or their waist-hip ratio. In contrast, the 24 women who could be characterized as perimenopausal, just starting the transition to menopause, demonstrated significant increases in the percent of their weight that was fat, increases in their waist circumference, and increases in their waist-hip ratio. The premenopausal women also had significant increases in the bone mineral density of the lumber spine, while those who were perimenopausal had significant bone loss, with a significant difference between the two groups. These differences indicate that changes in body composition and bone mass start before the cessation of menses, and that the early identification of women who will undergo these changes is possible.

Collaborators: M.C. Hochberg, M.D., M. Lethbridge-Cejku, Ph.D, and K. Martin, M.D., University of Maryland; M. Bellantoni, M.D., M. Blackman, M.D., and W.W. Scott, Jr., M.D., Johns Hopkins University; W. Horton, Ph.D, S.M. Harman M.D., R. Andres, M.D., D. Elahi, Ph.D., P. Costa, Ph.D, R. Hirsch M.D., NIH.

Josephine Egan, M.D.
Acting Chief, Diabetes Section

Gerontology Research Center
Room 2-B-01
Phone 410-558-8414
Fax 410-558-8381
E mail eganj@vax.grc.nia.nih.gov

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Recent Publications:

Wang Y, et al. *J Clin Invest* 1997; 99: 2883-2889.

Wang Y, et al. *Mol Cell Endocrinol* 1996; 116: 81-87.

Perfetti R, et al. *J Gerontol: Biological Sciences* 1996; 51: B331-B336.

Perfetti R, et al. *Am J Physiol* 1995; 269: B983-B990.

Biography: Dr. Josephine Egan received her M.D. from University College in Galway, Ireland in 1978. She is a board certified endocrinologist who received her endocrine training at the University of Virginia, Charlottesville. She has been at the GRC since July, 1990 and on the tenure track since July, 1994. Her early work from her fellowship related to investigating and quantitating insulin release from individual beta cells in the islets of Langerhans. Using this methodology, she outlined the abnormalities that occur in the aging beta cells of rats. More recently she has been working on ways to reverse these abnormalities and on ways to increase insulin secretion in Type II diabetes mellitus.

Aging and Type II Diabetes: The goal is to design new drugs to restore glucose sensitivity to the beta cells in Type II diabetes and to prevent deterioration of the beta cells which seems an inevitable occurrence in aging. The general strategy is to outline the abnormalities that occur in aging and Type II diabetes in beta cells and the search for agents that can alter these processes. The approach is to take the agents that have been first tested in beta cell lines into animal models of aging and diabetes, and with the information gained from the animal models, go as quickly as possible directly into the human situation.

Type II diabetes develops, for the most part, because insulin becomes less effective at its target tissues with increasing age, adiposity and changing lifestyle. This puts increased demand on the beta cells of the pancreas which then must supply more insulin. When supply cannot keep up with demand, blood sugars rise which then lead to complications such as blindness, nephropathy and neuropathy as a direct result of the elevated blood sugars. With increasing age, beta cells respond less well to glucose stimulus. They also do not replicate at the same rate as beta cells in younger animals. Thus, in principle, we need to find agents that would restore glucose responsivity to the beta cells and that would prevent the decrease in replication that occurs in aging mammals.

Design of Drugs of Potential Use in Type II Diabetes: We have been concentrating on a group of peptides known as incretins. They are released from the gut in response to food and they augment the insulin response to glucose. One of these peptides, GLP-1, is effective at increasing insulin release when given systemically even in long-standing Type II diabetes. It also appears to be a trophic agent to the pancreas in pharmacological doses. This is a major difference from other agents that are presently used to treat diabetes as studies show that even with good control of blood sugars, there is an inexorable decline in beta cell function. GLP-1 has a short half-life and consequently has to be given at least three times a day subcutaneously to maintain high insulin levels in the blood. We are presently working with a peptide called Exendin-4 which is secreted in the saliva of the Gila monster (a lizard) and which is 53% homologous to human GLP-1. It also is very effective at inducing insulin release and, of great significance, when given subcutaneously or intraperitoneally, it has a much longer biological action than GLP-1. We are presently involved in animal testing of this compound and hope to be involved in the human testing. We are also testing Exendin-4 that has been "humanized" i.e. we are replacing the amino acids of Exendin-4 with those of GLP-1 and hope to find out where the crucial amino acids that are responsible for the prolonged biological activity of Exendin-4 lie. Current efforts show that GLP-1 is a true growth factor for beta cells in the pancreas and perhaps is involved in cell differentiation in other organs besides pancreas.

Collaborators: Drs. Joel Habener, Doris Stoffers and Dariush Elahi, Massachusetts General Hospital; Drs. Seamus Shreenan and Anthony Pick, University of Chicago Medical School; Drs. Marie Byrne and Burkgard Goke, Marlburg, Germany; Dr. Nigel Greig, Laboratory of Cellular and Molecular Biology, NIA; Dr. Andrej Janczewski, Laboratory of Cardiovascular Sciences, NIA; Dr. Andrew Young, Amylin Pharmaceuticals, San Diego.

Michel Bernier, Ph.D.
Tenure-Track Investigator, Diabetes Section

Gerontology Research Center
Room 2-B-01
Phone 410-558-8199
Fax 410-558-8381
E mail bernierm@vax.grc.nia.nih.gov

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Recent Publications:

Kole HK, et al. *J Biol Chem* 1996; 271: 14302-14307.

Kole HK, et al. *J Biol Chem* 1996; 271: 31619-31626.

Perfetti R, et al. *Endocrinology* 1997; 138: 1829-1835.

Biography: Dr. Bernier received his Ph.D. from the University of Montreal, Canada, in 1983, and completed two postdoctoral fellowships. The first one was held in Unit 162 of the National Institutes of Health and Medical Research (INSERM) in Lyon, France, and the second one at the Johns Hopkins University School of Medicine in Baltimore. He was an assistant professor of Biochemistry at McGill University in Canada before coming to the NIA in 1990. His research interest concerns molecular aspects of insulin receptor signal transduction. He is a member of the American Diabetes Association.

Molecular Aspects of Insulin Receptor Signaling: Diabetes mellitus is one of the most prevalent illnesses among the elderly and is a comorbid condition in other diseases of the elderly. It is the hyperglycemia per se that leads to most of the complications of diabetes. The cause of diabetes mellitus is a diminished ability of the beta cells of the pancreas to release insulin in response to blood glucose, and a decreased insulin response at the target tissues. Treatments presently available to lower blood glucose are less than adequate. One goal of the Diabetes Section is to improve our understanding of factors regulating insulin action at the target cells in order to provide new insight into effective treatments for this and other metabolic-related diseases, including obesity.

There is a large body of evidence supporting the concept of glucose toxicity, in which hyperglycemia causes the deterioration of insulin secretion from the pancreas and insulin action in peripheral tissues (e.g., adipose, heart and skeletal muscle). This resistance to insulin action has been linked to an increase in glucose metabolism through the hexosamine biosynthetic pathway. Because the effect of high concentrations of glucose and glucose metabolites on the expression of genes important for regulation of adipose cell functions remains largely unknown, we have undertaken a study to look at the regulation of the transcription factor CCAAT/enhancer-binding protein (C/EBP) alpha by glucose and glucosamine, a product of the hexosamine pathway, in a cultured adipose

cell line that is known for its high responsiveness to insulin. This transcription factor has been implicated in the establishment and maintenance of energy homeostasis in adipocytes by controlling the expression of several gene products includeing the insulin-responsive glucose transporter GLUT4, lipid-synthesizing enzymes, and leptin. Our initial results indicate that the decrease in transcription and mRNA accumulation of the C/EBP-alpha gene caused by high concentrations of glucose or glucosamine is accompanied by a proportional reduction in the expression of the GLUT4 gene. We have also used a cell line that stably expresses a portion of the promoter for C/EBP-alpha gene fused to a reporter gene and demonstrated that promoter activity was decreased by these treatments. It is now recognized that the adipose tissue plays major endocrine roles in addition to its function as an energy storage depot. We are carrying out a study aimed at evaluating the effect of hyperglycemia or glucosamine on known molecular regulators of adipocyte metabolic and endocrine functions in rats. Progress in our understanding of factors regulating obesity-related insulin adipose cell physiology will allow the development of effective interventions for resistance, a major contributor to morbidity and mortality in the U.S. and other Western societies.

Despite close similarities in the structure of their receptors, insulin and insulin-like growth factor 1 (IGF-1) have different physiological functions. Differential interaction of the receptor intracellular domains with effector proteins may provide one mechanism by which insulin and IGF-1 signaling diverges. We have found that a discrete non-catalytic region of the carboxyl-terminal domain of the insulin receptor appears to contribute to the specific enhancement of insulin receptor activity, while having no effect on IGF-1 receptor function. Current efforts are concentrated on the characterization of the functional importance of this region of the insulin receptor in receptor signaling by using a minigene approach. The ability of this stably expressed receptor domain to enhance selectively insulin responsiveness with respect to receptor activation and function toward signaling intermediates and DNA synthesis will be evaluated. Another goal of this project is to further define the specific nature of insulin signaling regarding programmed cell death (apoptosis). Apoptosis is a natural phenomenon that plays a major role in normal turnover of cells. Insulin has been shown previously to rescue cells from apoptotic death. However, the concentrations of insulin used in these studies were supraphysiologic and could have exerted protection by activating the IGF-1 receptor, whose role as a survival factor has been well documented. Our preliminary experiments indicate that insulin possesses antiapoptotic properties but utilizes a signaling pathway that differs from that of IGF-1. This finding strengthens the notion that divergence in the signal cascade between these two hormones could originate from distinct intrinsic

properties of each receptor. A better understanding of the nature of these properties may represent a target for the development of selective receptor activator drugs. Protein tyrosine phosphatases (PTPases) have been found to dephosphorylate key tyrosyl residues from the insulin receptor kinase domain, thereby causing an inactivation of the receptor kinase activity and insulin action. Thus, PTPases may oppose tyrosine kinase-mediated insulin signaling and contribute to insulin resistance. Indeed, altered PTPase activity has been noted in different tissues from diabetic rats and in humans. Therefore, the development of PTPase inhibitors that can selectively block insulin receptor dephosphorylation might have therapeutic value. Our previous work has shown that a tris-sulfotyrosyl-containing peptide, 3S-peptide-I, whose primary sequence is identical to that of the kinase domain of the insulin receptor, exerts a potent inhibition against members of the PTPase family. In cultured cells that were semi-permeabilized, the addition of 3S-peptide-I was found to greatly reduce tyrosine dephosphorylation of preactivated insulin receptors, but not that of EGF receptors. We have also modified 3S-peptide-I by incorporating a stearyl moiety at the N-terminus of the peptide. This derivative has been shown to enhance insulin-stimulated receptor activation and signal transduction in intact cells. In contrast, ligand-stimulated EGF receptor functions were not affected by stearyl-3S-peptide-I. Thus, it appears that this peptide selectively enhances insulin signal transduction by specifically inhibiting dephosphorylation of the insulin receptor in intact cells. This result indicates that 3S-peptide-I or a derivative thereof may be a valuable tool in the identification, purification and characterization of PTPase(s) acting on the insulin receptor. Moreover, the administration of this or other related compounds in animal models of obesity and Type II diabetes would provide some insight into whether improvement of glucose homeostasis can be obtained. This research effort may help develop new agents that have potential therapeutic value for the treatment of diabetes.

Collaborators: Jeremy Tavare, Ph.D., University of Bristol, UK; Lance Macaulay, Ph.D., CSIRO Division of Biomolecular Engineering, Australia; M. Daniel Lane, Ph.D., Johns Hopkins University, Baltimore.

S. Mitchell Harman, M.D., Ph.D.
Chief, Endocrinology Section

Gerontology Research Center
Room 2-B-20
Phone 410-558-8186
Fax 410-558-8346
E mail mitch_harman@nih.gov

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Recent Publications:

Bellantoni MF, et al. *J Clin Endocrinol Metab* 1996; 81: 2848-2853.

Dobs AS, et al. *J Clin Endocrinol Metab* 1996; 81: 4108-4112.

Vittone J, et al. *Metabolism* 1997; 46: 89-96.

Biography: Dr. Harman is a 1970 graduate of the M.D., Ph.D. program at the State University of New York Health Sciences Center at Brooklyn. He trained in Internal Medicine at the Yale-New Haven Hospital, and in Endocrinology at National Institute of Child Health and Human Development (NICHD) as a Clinical Associate in the laboratory of Dr. Griff T. Ross. Dr. Harman is board certified in Internal Medicine and Endocrinology. He joined the Endocrinology Section, Laboratory of Clinical Physiology in 1974 (now Laboratory of Clinical Investigation), where he and his colleagues have helped elucidate the normal changes occurring with age in reproductive, growth, thyroid, and adrenal hormones and conducted investigations of hormone replacement in the elderly.

Changes in Hormone Regulation with Aging and Utility of Hormone Replacement Interventions: Research in the Endocrinology Section (ES) has documented alterations in hormone balance during the normal aging process by measuring changes in dynamic hormone secretory patterns in women and men using sensitive, state of the art methods. This work also explores the relationships of hormone secretion to states of nutrition and health and interrelationships among various hormones. Studies also elucidate the influence of alterations of endogenous hormone activity on risk factors for age-related chronic diseases. Finally, the ES conducts research on the clinical utility and risk/benefit ratios of hormone replacement interventions designed to reverse age-related alterations of hormone balance. The ES has maintained a close and consistent collaborative interaction with senior investigators at the Johns Hopkins University School of Medicine (Dr. Marc R. Blackman and Dr. Michele F. Bellantoni).

With aging, there are alterations in hormone secretion and in body composition. Loss of muscle mass may lead to reduced strength and functional capacity. Increased central fat may be associated with deterioration in lipid profiles and glucose tolerance (risk factors for heart disease). Aging is also associated with reductions in cardiac function and

fitness, immune function, and thinning of skin, all of which may have hormonal components. Decreases with age in the sex steroid hormones, testosterone (T) in men and estradiol (E_2) in women appear to play a role in the changes in body composition that occur with aging. Pituitary growth hormone (GH) decreases percent body fat and works via the intermediate hormone, insulin-like growth factor-I (IGF-I), to maintain calcium and nitrogen balance, and increase bone and muscle mass. Cortisol, the major steroid secreted by the adrenal cortex generally opposes the actions of GH and may increase slightly with age. Our work has demonstrated retention of a GH response to GH releasing hormone (GHRH), the brain peptide that stimulates GH secretion, an intact response of IGF-I to GH, and, more recently, improved muscle strength and lipoprotein patterns with no apparent changes in body composition, blood pressure, or glucose tolerance in men over 65 years of age treated with GHRH. Currently work examines the effects of hormone replacement for 26 weeks in healthy women and men >65 years old. Volunteers are randomized to treatment with GH, sex-appropriate steroid hormones, both GH and sex steroid, or placebos only and studied intensively at baseline and at 26 weeks. Studies include overnight blood sampling for GH, cortisol, and LH secretory profiles assessment of thyroid and reproductive hormones and leptin. Every 4 weeks, we measure glucose, CBC, and IGF-I, and serum T (men) or serum E_2 (women). Muscle-related endpoints include strength, muscle mass by magnetic resonance imaging (MRI), and muscle biopsies (for histology, histochemistry, and molecular responses). Additional endpoints include body composition defined by multiple procedures, whole body protein synthesis by ^{13}C-leucine uptakes, cardiovascular function and anatomy, and vascular reactivity. Bone metabolism is assessed from biochemical measures. Metabolic measurements include lipid profiles and glucose and insulin during an oral glucose tolerance test. Subsidiary studies examine immune function at baseline and in response to immunization and psychological function and quality of life. Because this large study remains in progress (100 subjects enrolled to date), treatment groups are still masked. Thus, analyses have been restricted to exploring relationships among variables at baseline. Mathematical analysis (deconvolution) of overnight secretory profiles reveals that secretion of cortisol appears to be directly proportional to secretion of GH. Because cortisol acts to break down lean tissue and bone and GH to build them up, the observation that their secretory rates are linked suggests the presence of a protective compensatory mechanism in the elderly to keep these opposing hormone influences in balance. We also find that plasma levels of leptin, the fat cell hormone that inhibits appetite, is directly and independently related to adiposity (% body fat) rather than to age, sex or levels of other hormones. Thus, leptin may serve as a biomarker of total adiposity in elderly, as well as young women and men.

The ES also participates as an active contributor to ongoing studies of estrogen replacement therapy in women, longitudinal assessments of the physiology of the perimenopause, and longitudinal studies of testosterone and other steroid hormones in aging men and their relationship to prostate disease. These investigations are carried out in collaboration with other intramural investigators (Dr. Metter, and Dr. Tobin, LCI) and with extramural investigators (Dr. Blackman, Medicine, JHU and Dr. Bellantoni, Geriatric Medicine, JHU).

Future research will examine the effects of augmenting GH secretion with GH releasing peptide (GHRP), a secretagogue which produces a more physiologic pattern of GH secretion than does GH treatment. Studies will examine the interaction of GHRP and sex steroids on bone in women and men with osteoporosis and the responses of normal and failing hearts in older patients to GHRP intervention. A collaborative study with the Laboratory of Clinical Immunology examining effects of DHEA (dehydroepiandrosterone) on T- and B-cell responses to immunization in the elderly is also planned.

Collaborators: Marc R. Blackman, M.D.; Michele F. Bellantoni, M.D., Jocelyn Jayme, M.D., Johns Hopkins University; Kieran O'Connor, M.D., Endocrinology Section, LCI, NIA; Jordan Tobin, M.D., Applied Physiology Section, LCI, NIA; Jeffrey Metter, M.D., Longitudinal Studies Section, LCI, NIA; Lawrence Jacobs, M.D., University of Rochester School of Medicine.

James L. Fozard, Ph.D.
Chief, Longitudinal Studies Section

Gerontology Research Center
Room 3-A-06
Phone 410-558-8364
Fax 410-558-8321
E mail fozardj@grc.nia.nih.gov

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Recent Publications:

Deeg DDH, et al. *Handbook of the Psychology of Aging* 1996; 129-149.

Metter EJ, et al. *J Gerontol: Biol. Sci* 1997; 52: B267-B276.

Carter HB, et al. *JAMA* 1997; 277: 1456-1460.

Morrell C, et al. *J Acoust Soc Am* 1996; 100: 1949-1967.

Biography: James L. Fozard received his Ph.D. in Experimental Psychology from Lehigh University in 1961 and completed a two year postdoctoral fellowship at the Massachusetts Institute of Technology in 1966. From 1967 to 1978 he was a research psychologist with the Department of Veterans Affairs Normative Aging Study, Co-director of the Boston Division of the Boston/Bedford Geriatric Research, Educational and Clinical Center (1976-1978) and a member of the Psychiatry Department Faculty at Harvard Medical School (1968-1979). From 1978 to 1985, he was the Director of the Patient Treatment Service in the Office of Geriatrics and Extended Care, Department of Veterans Affairs. He joined the NIA in 1985 as Head of the Baltimore Longitudinal Study of Aging.

Prostate Aging and Disease: This BLSA project has a retrospective arm and a prospective arm that is scheduled to run through 2003.The goal of the project is to characterize normal aging in the prostate and to identify transitions to prostate disease, particularly benign prostatic hyperplasia (BPH) and prostate cancer. In addition to evaluating hypotheses about the natural history of prostate aging and disease, the goal of the research is to use information about structure and function of the prostate for early detection of prostate disease. Clinical evaluations of prostate growth and function have been made in over 800 men with and without prostate disease and the availability of stored sera and genetic material permits analysis of known and newly developing risk factors. The prospective arm of the study consists of the men currently in the BLSA plus those ranging in age from 30 to 79 who enter the BLSA through 2003.

Baseline demographic information includes age, race, and information on variations in male hormone levels. Longitudinal data on male hormone levels is obtained from stored sera. In FY1997, all usable data on several male hormones has been placed into a computer-based file and initial analyses have begun. Sera from the prospective part of the study are frozen and stored and will be assayed as funds allow.

Prostate growth information in the retrospective part of the study comes from serial analyses of changes in serum levels of prostate specific antigen (PSA) and clinical evaluations including digital rectal examinations (DREs). In the prospective part of the study growth is also being assessed with magnetic resonance imaging (MRIs). To date, the major accomplishments have come from analyses of PSA levels which show that PSA increases linearly a bit faster over a period of years in men who develop BPH than in those who do not. The rate of change is still greater in men who develop prostate cancer, and the increases go up exponentially 5-7 years before diagnosis. Papers published in FY 1997 further show that when the ratio of free to total PSA is computed, the ability to distinguish changes in PSA levels between men who develop prostate cancer and those who do not increases from 4-5 years to about 10 years prior to diagnosis. Analyses of a subset of the men who developed prostate cancer show that the ratio is lower in men who have clinically defined aggressive tumors. Thus, rate of PSA increase (>0.75 units/year) identifies those likely to have prostate cancer and a low percentage of free PSA identifies those with aggressive disease whose cancer needs aggressive treatment.

Alterations in prostate structure or function are studied in relation to the possible development of prostate disease, particularly BPH. Currently, the MRI data are being analyzed to estimate prostate volume as well as the percentage of epithelial and stromal tissue. Symptoms associated with BPH are assessed with the standard AUA symptom questionnaire and with measures of urine flow and post-void residuals. Current analyses of cross sectional data indicate that, as expected, flow rate decreases with older age and that the distribution of positive responses to questionnaire queries about urinary symptoms increases. Except for limited symptom questionnaire data from the retrospective arm of the study, all of these measurements were initiated in the prospective arm of the study.

Diagnosis and tracking of prostate disease is based on current clinical practice. In the prospective arm of the study, participants who require further clinical evaluation and prostate biopsy are offered that evaluation free of charge at the Johns Hopkins Department of Urology.
We attempt to obtain all pertinent medical records about diagnoses and treatment of prostate disease. In the prospective part of the study we attempt to obtain and preserve the prostate of deceased participants on whom an autopsy was performed.

Genetic factors contributing to prostate disease are being studied. Starting in FY1997 a case-control study of four genes that may contribute to prostate cancer began. BLSA men who have prostate cancer are compared to age matched controls that, on the basis of longitudinal clinical observation, were judged to have a low probability of having prostate

cancer. The four genes are: the mu-class glutathione S-transferase (GST) gene, GSTM1; the pi-class GST gene GSTP1; the human androgen receptor gene hAR; and the inherited prostate cancer susceptibility gene PRCA.

A study of familial genetics in BPH is under review. The experimental subjects, BLSA participants with early onset BPH, will be identified on the basis of MRI and/or PSA data; first degree relatives will be studied to identify genes linked to prostate growth.

Hearing and Aging: Assessments of hearing thresholds for pure tones in consenting BLSA participants using a Bekesy audiometer began in 1965. An expanded hearing protocol was introduced in 1991 that included pure tone audiometry using more contemporary psychophysical procedures, acoustic reflex measures, speech perception in noise and a self-report of hearing difficulties. It continued until 1995 when it was necessary to discontinue the procedure after loss of the technician. A proposal to resume the protocol with an additional assessment of vestibular function is under review at present. The proposed protocol would be used with BLSA participants who have consented to be part of the autopsy study. There is considerable interest in studying the pathology of the auditory system in relation to hearing performance. Current activity is directed at analyzing and reporting the data from the expanded hearing protocol.

The published results of the BLSA longitudinal data on pure tone audiometry have become standard reference data. Data published in FY1997 provide longitudinal and cross-sectional percentiles of age-related changes and differences for men and women ranging in age from the 20s to the 80s. These data are valuable to persons concerned with assessing the effects of long term noise exposure on hearing of persons of different ages. Noise-induced hearing loss produces hearing loss greater than one would expect on the basis of aging alone and the projections allow one to estimate the excess hearing loss associated with noise exposure or other exogenous factors that would contribute to hearing loss.

The analyses of the data on self-reported hearing complaints (Hearing Handicap Inventory) indicate that the number of difficulties in hearing increase with aging as would be expected. However, when individual differences in pure tone hearing thresholds are taken into account, the importance of age as such is considerably diminished. The same is true when considering the relationship between self-reported hearing loss and speech perception and noise. Both are imperfectly correlated with age, and when all are considered together, the major factors determining self-reported hearing difficulties is hearing loss, not aging.

The analyses of the acoustic reflex data show that middle ear disorders are a minor contributor to the age associated changes in hearing that have been found. This result is consistent with earlier reports on small samples.

Collaborators—Prostate Aging and Disease: E.J. Metter, L.J. Brant, R. Andres, S.M. Harman, NIA; H. B. Carter, A.W. Partin, J.I. Epstein, D.W. Chan, P.C. Walsh, W. Isaacs, M. Blackman, Johns Hopkins Univ.; Joan Bailey-Wilson, NCGR; J.D. Pearson, H. Guess, Merck Laboratories.

Collaborators—Hearing and Aging: E.J. Metter, L.J. Brant, Kaori Nagai, NIA; Sandra Gordon-Salant, University of Maryland; C.H. Morrell, Loyola College, Baltimore, MD; J. D. Pearson, Merck Laboratories.

E. Jeffrey Metter, M.D.

Medical Officer, Baltimore Longitudinal Study of Aging

Gerontology Research Center
Room 3-A-08
Phone 410-558-8542
Fax 410-558-8321
Email metterj@grc.nia.nih.gov

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Recent Publications:

Kawas C, et al. *Neurology* 1997; 48: 1517-1521.

Stewart K, et al. *Neurology* 1997; 48: 626-632.

Carter HB, et al. *Urology* 1997; 49: 379-384.

Pearson JD, et al. *Urology* 1996; 48(6A): 4-9.

Biography: Dr. E. Jeffrey Metter received his M.D. from the University of California, Los Angeles in 1971. He completed a medical internship and neurology residency at the Mayo Graduate School of Medicine, Rochester, Minnesota in 1976. He returned to Los Angeles, where he became a staff neurologist and chief of the stroke rehabilitation ward at the Veterans Administration Medical Center, Sepulveda, California. He was also on the full time faculty in the Department of Neurology, UCLA School of Medicine. In 1987, he joined the National Institute on Aging as the physician for the Baltimore Longitudinal Study of Aging.

Health Evaluation in the Baltimore Longitudinal Study of Aging (BLSA): A clinical evaluation unit, under Dr. Metter's supervision, is responsible for the health evaluations in the BLSA. The characterization of the health status of all subjects is important to many of the researchers and projects within the study. Starting in 1985, the BLSA health evaluation has undergone major changes to improve medical information collection. The most substantial change occurred between 1988 and 1990, when the BLSA

initiated extensive use of nurse practitioners and physician assistants (NP/PA) to perform the history and physical examinations, rather than medical staff fellows. Subsequently, revisions have occurred in health questionnaires, medication and diagnosis listing.

We continually try to improve the quality of the clinical evaluation. We continue to assess quality assurance across the questionnaires, maintain staff training, and monitor and improve staff cooperation so that reliability and consistency of the clinical evaluation remains at a high level over time. This effort seems successful as over the past several years, staff has turned over, and new staff have easily adjusted and adapted to the unique needs of the BLSA. As new research questions are developed by scientific staff, we add new dimensions to the evaluation. We try to do this so those existing questionnaires are not changed, to maximize the longitudinal capabilities of the health data.

The unit is also responsible for the day to day health requirements of the participants during their visit. The unit tries to maintain and improve as necessary the high level of nursing and technical support, and to maximize the good will between the staff and the BLSA participants. The technical support includes health screening for a number of research protocols and assisting researchers in project development as it applies to unit interaction with the research. To meet these ends, the NP/PA and nursing staff have established quality assurance in the evaluation program. They have regularly scheduled meetings to discuss evaluation problems and related issues. A protocol manual was prepared describing most of the procedures and questionnaires. Ongoing efforts are designed to maximize the participant well-being, with continued monitoring of forms, records, protocols and comments of research participants.

Neuromuscular Changes with Age: The purpose is to characterize and explain age-associated losses of muscle strength. We seek to understand the time course of strength loss, factors that contribute to the loss, and to what degree the exercise response differs between old and young individuals. Our research has three main components.

1. Characterization of longitudinal strength changes in the BLSA. This consists of two parts. From 1960 to 1985, strength and power were measured in BLSA participants using in-house constructed equipment that measured isometric strength and power in the upper extremities. The purpose was to determine long term longitudinal changes (up to 25 years) in strength and power, and to relate these changes to changes in muscle mass, peripheral nerve function, daily and physical activity, and aerobic fitness. Starting in 1992, strength has been measured using a state of the art isokinetic dynamometer (Kin-Com). This equipment allows for the

measurement of both concentric and eccentric strength at multiple velocities in both the upper and lower extremities. The specific purposes are to determine age-associated maximal force production of the upper and lower body musculature during the concentric and eccentric phases of exertion, at fast, slow and zero speed, and determine the angle of greatest force; determine relationships between changes in strength with age and changes in lean body mass, fat mass, bone mineral density, glucose homeostasis, functional abilities, physical activity and nutritional state.

2. Comparison of exercise response to resistive strength training in young and old subjects. This project is being completed under contract with the University of Maryland, College Park, Dr. Ben Hurley, principal investigator. The specific purposes are: (1) determine the relationship between changes in lean body mass or muscle mass and changes in glucose regulation with age and strength training. (2) To determine if changes in strength or muscle mass can predict changes in total or regional bone mineral density. (3) To determine what factors best explain strength losses associated with aging and detraining and strength gains associated with strength training.

3. Examination of the motor unit and its relationship to muscle strength and exercise response. A protocol has been developed that explores motor unit function at different levels of muscle exertion in the quadriceps. The goal of this project is to understand the changes that occur in motor units with aging, and the effects of these changes on muscle strength and how these changes affect the exercise response. Over the past 20 years, *in vivo* techniques have allowed for the direct examination of the motor units in humans. Most studies that have examined age-related changes in motor units have focused on old versus young rather than examining the entire adult life span. They do not allow for an assessment of where during the life span these changes begin, or the association between the motor units and strength. The ongoing study addresses these issues.

Race and Gender Differences in Intracerebral and Carotid Arterial Velocity with Aging: This project is studying intracerebral blood flow velocity and resistance, and carotid blood flow velocity using doppler ultrasonographic techniques in BLSA participants. The goal is to determine whether differences in these parameters may explain racial and gender differences in stroke and coronary heart disease, and whether changes in arterial characteristics are associated with fitness and frailty.

We have found that intimal-media thickness of the common carotid artery increases with age concomitant with dilatation. Changes in wall thickness are associated with increasing risk for the development of coronary heart disease after adjusting for age. In addition, age changes in flow velocities in the carotid artery are poorly correlated with the flow velocities in the

middle cerebral arteries. We have also compared different measures of arterial stiffness across age and explored which measures are most related to the development of coronary heart disease. The development of a noninvasive technique to assess vascular stiffness, a major determinant of cardiovascular disease risk, may be valuable for identifying the effects of interventions aimed at modifying cardiovascular risk.

Collaborators - Neuromuscular Changes with Age: Robin Conwit, M.D., Johns Hopkins Bayview Medical Center; William Brown, M.D., Tufts University; Daniel Stashuk, Ph.D., University of Waterloo, Ontario, Canada; Benjamin Hurley, Ph.D., University of Maryland, College Park.

Collaborators - Race and Gender Differences in Intracerebral and Carotid Arterial Velocity with Aging: Christopher Earley, M.D., Ph.D., Johns Hopkins Bayview Medical Center.

Reubin Andres, M.D.
Chief, Metabolism Section

Gerontology Research Center
Room 2-B-13
Phone 410-558-8193
Fax 410-558-8113
E mail andresr@vax.grc.nia.nih.gov

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Biography: Dr. Andres received his medical degree and residency training at Southwestern Medical College in Dallas. His postdoctoral fellowship began at Johns Hopkins in 1950 and he has maintained his academic appointment there as Professor of Medicine. He came to the NIH in 1962 to be the Clinical Director and Assistant Chief of the Gerontology Unit in Baltimore, initially when it was in the National Heart Institute, then in the National Institute of Child Health and Human Development, and now in NIA. Dr. Andres is past president of the Gerontological Society, a member of the American Society of Clinical Investigators and the Association of American Physicians, and the recipient of the Kleemeier Award, the Allied-Signal Achievement Award in Aging, the Enrico Greppi Gerontology Prize (Italy), and the Rank Prize in Nutrition.

Recent Publications:

Muller DC, et al. Muscle Mass: Its Measurement and Influence on Aging. The BLSA. *Nutrition Assessment of Elderly Populations* 1995; pp. 50-62.

Andres R, et al. Body Weight and Age. *Comprehensive Textbook of Eating Disorders and Obesity* 1995; pp. 65-70.

Muller DC, et al. *Aging Clin Exp Res* 1996; 8: 13-21.

Edelstein S, et al. *Diabetes* 1997; 46: 701-710.

Glucose/Insulin Homeostasis and Aging: Several diverse research approaches are in progress in order to understand the role of aging in the progressive changes occurring in this complex metabolic axis. (1) Factors influencing the age changes in fasting glucose and in glucose tolerance have been shown to be obesity and a central pattern of fat deposition, physical inactivity, dietary variables, physical inactivity, and a number of distinct diseases and medications associated with aging. (2) The glucose clamp technique (hyperglycemia and hyperinsulinemic/euglycemic) was devised in order to quantify, in intact humans, (a) beta cell responsiveness to glucose and to incretins (GIP and GLP) and (b) sensitivity of body tissues to insulin. (3) The implications of elevated fasting glucose and glucose tolerance values for the development of the characteristic complications of diabetes are being quantified in participants in the Baltimore Longitudinal Study of Aging. The development of coronary artery disease, the overt diabetic state, and all-cause mortality are under study. (4) The diagnostic cutpoints for the "impaired" state and for diabetes, recently recommended by the American Diabetes Association, are being carefully examined with reference to the possibility that an adjustment might be required for older men and women. Data from the BLSA, the Rancho Bernardo Study, and the National Health and Nutrition Examination Survey III are being collated.

Interactions of Aging, Obesity, and Mortality: There is continuing controversy over recommended weight-for-height in men and women and whether or not these standards need to be age-specific. The NHANES I Follow-up Study provides an unparalleled data set to examine the association between Body Mass Index at age 55-74 years at entry being and subsequent mortality over the next 20 years in white and black men and women. In addition, collaboration with the Applied Physiology Section, some 40 years of anthropometric measurements have been used to generate equations for the computation of percent body fat using DEXA scanning as the gold standards.

Collaborators: Dr. Dariush Elahi, Massachusetts General Hospital; Dr. Elizabeth Barrett-Connor, University of California, San Diego; Dr. Katherine Flegal, National Center for Health Statistics; Drs. John Sorkin and Andrew Goldberg, University of Maryland; Dr. Jordan Tobin, Applied Physiology Section, LCI, NIA; Dr. Josephine Egan, Diabetes Section, LCI, NIA; Dr. Ballentine Carter, Johns Hopkins; Dr. Judith Hallfrisch, Beltsville Human Nutrition Research Center, USDA; Dr. Katherine Tucker, Human Nutrition Research Center, Tufts University.